FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)
05118.0002U2	10/009359

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/12953	12 MAY 2000	13 MAY 1999

TITLE OF INVENTION

PRODUCTION OF HUMAN CELLS, TISSUES, AND ORGANS IN ANIMALS

APPLICANT(S) FOR DO/EO/US

TOWNES et al. Tim M.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5))

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter
19. ☒ Certificate of Mailing by Express Mail — Express Mail No. EL924206202US dated November 13, 2001
20. ☒ Other items or information:

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EL924206202US

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/009359	PCT/US00/12953	05118.0002U2

21. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1,000.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO **$860.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**	
☒	International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**	
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	
	ENTER APPROPRIATE BASIC FEE AMOUNT =	**$710.00**
	Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$130.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	19 - 20 =	0	x $18.00	**$0.00**	
Independent claims	4 - 3 =	1	x $84.00	**$84.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
	TOTAL OF ABOVE CALCULATIONS =			**$924.00**	
	Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable).** ☒			**$462.00**	
	SUBTOTAL =			**$462.00**	
	Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +			**$0.00**	
	TOTAL NATIONAL FEE =			**$462.00**	
	Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐			**$0.00**	
	TOTAL FEES ENCLOSED =			**$462.00**	
				Amount to be: refunded	$
				charged	$

☒ A check in the amount of **$462.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **14-0629** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

David G. Perryman, Esq.
NEEDLE & ROSENBERG, P.C.
127 Peachtree Street, N.E.
Suite 1200
Atlanta, GA 30303-1811

SIGNATURE

David G. Perryman
NAME

33,438
REGISTRATION NUMBER

13 NOVEMBER 2001
DATE

ATTORNEY DOCKET NO. 05118.0002U2
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
	)	
TOWNES *et al.*	)	
	)	Group Art Unit: Unassigned
Serial No. Unassigned	)	
	)	Examiner: Unassigned
Filed: Herewith	)	
	)	
FOR: "PRODUCTION OF HUMAN CELLS, TISSUES, AND ORGANS IN ANIMALS"	)	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Box PCT (IPEA/US)
Washington, D.C. 20231

NEEDLE & ROSENBERG, P.C.
Suite 1200, The Candler Building
127 Peachtree Street, N.E.
Atlanta, Georgia 30303-1811

November 13, 2001

Sir:

Prior to the issuance of an Office Action pertaining to the above-identified patent application, please enter the following preliminary amendment and consider the following remarks. A copy of the marked-up claims is attached as Appendix A to this Amendment.

IN THE CLAIMS

Please rewrite the following claims.

9. The method of claim 6, wherein the cloning cell is an embryonic, fetal, or adult fibroblast.

10. The method of claim 6, wherein the reprogramming cell is an unfertilized enucleated egg from another organism of the same, or a different, species from which the cloning cell was obtained.

11. The method of claim 6, wherein the function of the gene is knocked out by homologous recombination.

12. The method of claim 6, wherein the nuclear genetic material of the cloning cell is introduced into the reprogramming cell by nuclear transfer.

16. A method of treating a patient in need of a transplant of a cell, tissue, organ, comprising transplanting into the patient cells, a tissue, or an organ produced in the animal of claim 1.

17. A cell, tissue, or organ produced by the method of claim 6.

18. Use of a cell, tissue, or organ produced by the method of claim 6 in the treatment of a patient in need of a transplant of a cell, tissue, or organ, by transplanting the cell, tissue, or organ into the patient.

19. Use of A cell, tissue, or organ produced by the method of claim 6 in preparation of a medicament for treatment of a patient in need of a transplant of the cell, tissue, or organ.

IN THE SPECIFICATION

On page 1 of the specification, before the first paragraph, please insert the following:

-- The present application is a 35 U.S.C. § 371 national phase application from, and claims priority to, international application PCT/US00/12953, filed May 12, 2000 (published under PCT Article 21(2) in English), which claims priority to U.S. provisional patent application Serial No. 60/133,935, filed May 13, 1999, which applications are hereby incorporated herein in their entirety.--

REMARKS

Claims 9-12 and 16-19 have been amended herein to remove the multiple dependency. This amendment neither narrows the scope of the claim nor was it made for reasons related to patentability.

The specification is amended herein to update the priority claim for this application. It is believed that no new matter has been added by this amendment, and applicants respectfully request entry of same into the present application.

No fee is believed due; however, the Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

David G. Perryman
Registration No. 33,438

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CERTIFICATE OF EXPRESS MAILING

I hereby certify that this correspondence along with anything indicated as being attached or included is being deposited with the United States Postal Service as Express Mail No. EL924206202US in an envelope addressed to: Assistant Commissioner for Patents, Box PCT (IPEA/US), Washington, D.C. 20231, on the date shown below.

Erick Calderon

11/13/01
Date

Appendix A
Marked-up claims

9. (Amended) The method of claim 6[, 7, or 8], wherein the cloning cell is an embryonic, fetal, or adult fibroblast.

10. (Amended) The method of claim 6[, 7, or 8], wherein the reprogramming cell is an unfertilized enucleated egg from another organism of the same, or a different, species from which the cloning cell was obtained.

11. (Amended) The method of claim 6[, 7, or 8], wherein the function of the gene is knocked out by homologous recombination.

12. (Amended) The method of claim 6[, 7, or 8], wherein the nuclear genetic material of the cloning cell is introduced into the reprogramming cell by nuclear transfer.

16. (Amended) A method of treating a patient in need of a transplant of a cell, tissue, organ, comprising transplanting into the patient cells, a tissue, or an organ produced in the animal of claim 1[or by the method of claim 6, 7, or 8].

17. (Amended) A cell, tissue, or organ produced by the method of claim 6[, 7, or 8].

18. (Amended) Use of a cell, tissue, or organ produced by the method of claim 6[, 7, or 8] in the treatment of a patient in need of a transplant of a cell, tissue, or organ, by transplanting the cell, tissue, or organ into the patient.

19. (Amended) Use of a cell, tissue, or organ produced by the method of claim 6[, 7, or 8] in preparation of a medicament for treatment of a patient in need of a transplant of the cell, tissue, or organ.

13 Rec'd PCT/PTO 13 NOV 2001
PCT/US00/12953
10/009359

PRODUCTION OF HUMAN CELLS, TISSUES, AND ORGANS IN ANIMALS

Background of the Invention

This invention relates to animals that produce cells, tissues, and organs of another organism (*e.g.*, a human), and methods of generating such chimeric animals. In brief, non-functional cells, tissue(s), or organ(s) of one animal species are replaced with functional cells, tissue(s), or organ(s) from a second species by creating a chimeric animal. Potentially any cell type, tissue, or organ of an animal is amenable to this process.

Everyday, thousands of people of all ages are admitted to hospitals because of the malfunction of a vital organ and, because of a lack of organs available for transplantation, many of these people will die. Immunological incompatibility between patients and donated organs increases the possibility that, even if an organ is available, it cannot be used for a particular patient in need. Moreover, even for those patients fortunate enough to receive a donated organ, life-long administration of immunosuppressants may be required. The risk of a donated organ containing human pathogens adds another dimension to this problem.

The present invention removes both of these risks, because the donated cells, tissue(s), or organ(s) that are grown in the chimeric animal can be derived from an afflicted individual, and, thus, the transplant can be autologous.

Cloning, or nuclear transfer, is a method in which nuclear genetic material is taken from a differentiated cell of an organism and is transferred into a "reprogramming cell," which is typically an unfertilized, enucleated egg of another organism of the same species. The nucleus of this reconstructed cell dedifferentiates into a totipotent progenitor that is implanted into the uterus of a foster mother, where it develops into an organism having the same genetic makeup as the organism from which the original nuclear genetic material was derived. Additionally, cells from an organism that have been genetically modified *in vitro* can be reprogrammed to produce a clone of the original organism that contains the precise genetic modification engineered *in vitro*. Thus, cloning makes possible the generation of multiple identical organisms that contain precise genetic modifications (see, *e.g.*, Campbell *et al.*,

Nature 385:810-813, 1997: Wilmut, Scientific American, 58-63, December, 1998; Cibelli *et al.*, Science 280:1256-1258, 1998).

Summary of the Invention

In general, the invention provides animals that produce cells, tissue(s), or organ(s) of another organism (*e.g.*, a human), and methods of making such animals. Cells, tissue(s), or organ(s) produced in the animals of the invention can be taken from the animals and transplanted into patients in need of such cells, tissue(s), or organ(s).

Accordingly, in a first aspect, the invention features an animal (or precursor thereof) that contains cells, tissue(s), or organ(s) (or precursor thereof) of another organism (*e.g.*, a human), but not the corresponding cells, tissue(s), or organ(s) (or precursor thereof) that would otherwise naturally occur in the animal. The animal can be, for example, a cow, a sheep, a pig, a mouse, or a primate, such as a chimpanzee, monkey, or ape. The cells can be, *e.g.*, red blood cells, pancreatic islet cells, epithelial cells, neurons, or chondrocytes; the tissue(s) can be, *e.g.*, blood, the retina, or cartilage; and the organ(s) can be, *e.g.*, a pancreas, a heart, a liver, a kidney, intestine, a lung, or skin. Genes that can be knocked out to generate the animals of the invention include, for example, GATA-2 (blood), LMO-2 (blood), globin genes (*e.g.*, α-globin and β-globin; blood), the erythropoietin receptor gene (erythroid cells), PDX-1 (pancreas), and IPF-1 (Insulin promoter factor-1; pancreas).

In a second aspect, the invention features a method of generating an animal, as described above, which has cells, tissue(s), or organ(s) of another organism, but not the corresponding cells, tissue(s), or organ(s) that would otherwise naturally occur in the animal. In this method, the function of a gene that is required for development of the cells, tissue(s), or organ(s) is knocked out in a cell (*e.g.*, an embryonic, fetal, or adult fibroblast) of an animal, for example, by homologous recombination, to generate a genetically modified "cloning cell." Nuclear genetic material of the cloning cell, or a derivative thereof, is then introduced (by nuclear transfer) into a "reprogramming cell" (typically an unfertilized, enucleated egg) from another organism of the same (or different) species from which the cloning cell was obtained. Alternatively, the cloning cell is fused with the reprogramming cell. In either case the resulting reconstructed cell is stimulated to develop into an embryo. When this embryo reaches the blastocyst stage of development, "donor" embryonic stem cells of another organism (*e.g.*, a human) are introduced. The resulting blastocyst is implanted into a

pseudopregnant foster mother, where it develops into a chimeric animal that produces cells, tissue(s), or organ(s) derived from the donor embryonic stem cells, but not the corresponding cells, tissue(s), or organ(s) that would otherwise naturally occur in the animal. The cells, tissue(s), or organ(s) that develop from the implanted donor embryonic stem cells correspond to those cells, tissue(s), or organ(s) that are absent in the developing embryo/fetus because the gene (or genes) required for normal development and/or differentiation were knocked out in the cloning cells.

In a third aspect, the invention features a method of generating an animal, as described above, which has cells, tissue(s), or organ(s) of another organism, but not the corresponding cells, tissue(s), or organ(s) that would otherwise naturally occur in the animal. In this method, as described above, the function of a gene that is required for development of the cells, tissue(s), or organ(s) is knocked out in a cell (*e.g.*, an embryonic, fetal, or adult fibroblast) of an animal, for example, by homologous recombination, to generate a genetically modified cloning cell. Nuclear genetic material of the cloning cell, or a derivative thereof, is then introduced (by nuclear transfer) into a reprogramming cell (typically an unfertilized, enucleated egg) from another organism of the same (or different) species from which the cloning cell was obtained. Alternatively, the cloning cell is fused with the reprogramming cell. In either case, the resulting reconstructed cell is stimulated to develop into an embryo that is implanted into a pseudopregnant foster mother. At the appropriate developmental time, "donor stem cells" (*e.g.*, hematopoietic stem cells) of another organism (*e.g.*, a human) are surgically introduced into the developing embryo and/or fetus *in utero*, and the resulting fetus develops into a chimeric animal that produces cells, tissue(s), or organ(s) derived from the donor stem cells, but not the corresponding cells, tissue(s), or organ(s) that would otherwise naturally occur in the animal. The cells, tissue(s), or organ(s) that develop from the implanted donor stem cells correspond to those cells, tissue(s), or organ(s) that are absent in the developing embryo/fetus because the gene (or genes) required for normal development and/or differentiation were knocked out in the cloning cells.

In a fourth aspect, the invention features a method of generating an animal, as described above, which has cells, tissue(s), or organ(s) of another organism, but not the corresponding cells, tissue(s), or organ(s) that would otherwise naturally occur in the animal. In this method, as described above, the function of a gene that is required for development of the cells, tissue(s), or organ(s) is knocked out in a cell (*e.g.*, an embryonic, fetal, or adult fibroblast) of an animal, for example, by homologous

recombination, to generate a genetically modified cloning cell. Nuclear genetic material of the cloning cell, or a derivative thereof, is then introduced (by nuclear transfer) into a reprogramming cell (typically an unfertilized, enucleated egg) from another organism of the same (or different) species from which the cloning cell was obtained. Alternatively, the cloning cell is fused with the reprogramming cell. In either case, the resulting reconstructed cell is stimulated to develop into an embryo. When the embryo has developed to the morula stage (4 to 16 cell stage) the individual blastomeres are disaggregated. Chimeric morula are constructed by injecting the above disaggregated blastomeres along with disaggregated donor blastomeres of another organism (*e.g.*, a human) back into the zona pellucida or by aggregating the blastomeres of the two organisms. These reconstructed morula are implanted into a pseudopregnant foster mother, where they develop into chimeric animals that produce cells, tissue(s), or organ(s) derived from the donor blastomeres, but not the corresponding cells, tissue(s), or organ(s) that would otherwise naturally develop in the animal. The cells, tissue(s), or organ(s) that develop from the implanted donor blastomeres correspond to those cells, tissue(s), or organ(s) that are absent in the developing embryo/fetus because the gene (or genes) required for normal development and/or differentiation were knocked out in the cloning cells.

In a fifth aspect, the invention provides methods of treating patients in need of a transplant of a particular cell type, tissue, or organ, by introducing into the patient appropriate cells, a tissue, or organ produced in an animal of the invention, as described above. Also included in the invention is the use of cells, tissues, or organs produced in the invention in the treatment of disease.

The invention provides several advantages. For example, cells, tissue(s), or organ(s) produced using the methods of the invention can be grown from cells having the nuclear genetic material of a person into whom the cells, tissue(s), or organ(s) are to be ultimately transplanted, thus eliminating problems associated with adverse immunological reactions and the need for prolonged use of immunosuppressants in transplant recipients. Also, since the human cells, tissue(s), or organ(s) of the invention are produced in an animal, the cells, tissue(s), or organ(s) are free of human viruses and other pathogens, such as human immunodeficiency virus (HIV). Other features and advantages of the invention will be apparent from the following detailed description.

Detailed Description

The invention provides animals (*e.g.*, mice, cows, sheep, pigs, and primates (*e.g.*, chimpanzees, monkeys, and apes)) that produce, cells, tissue(s), or organ(s) of another organism (*e.g.*, a human), but not corresponding cells, tissue(s), or organ(s) that would otherwise naturally occur in the animals. For example, animals provided in the invention include a cow that produces human, but not bovine, red blood cells; a cow that produces human, but not bovine, blood; and a cow that produces a human, but not a bovine, pancreas. The invention also provides methods of making such animals.

The animals of the invention are made using either of three general strategies. In the first strategy, the function of a gene (or genes) necessary for the production of a particular cells, tissue(s), or organ(s) in an animal is knocked out in a cultured cell of the animal, and the nuclear genetic material of this so-called "cloning cell" (or a derivative of this cell) is introduced, by nuclear transfer, into a so-called "reprogramming cell," which is, typically, an unfertilized, enucleated egg of an animal of the same (or different) species as the animal from which the cloning cell is derived. After nuclear transfer, the resulting egg is stimulated (*e.g.*, electrically or chemically) to develop, grown into a blastocyst, into which human embryonic stem cells are introduced. The introduced human cells are out-competed in the development of all cells, tissue(s), or organ(s) of the animal, except for the cells, tissue(s), or organ(s) dependent for development on the gene (or genes) that was knocked out in the cloning cell (or its ancestor) used in the nuclear transfer. Thus, an animal that develops from this blastocyst contains normal cells, tissue(s), or organ(s) of the animal from which the cloning cell was derived, except for the cells, tissue(s), or organ(s) dependent for development on the knocked out gene (or genes); for these cells, tissue(s), or organ(s), the animal contains cells, tissue(s), or organ(s) corresponding to the organism (*e.g.*, a human) from which the embryonic stem cells that were introduced into the blastocyst were derived.

The second and third strategies differ from the first strategy only in the type of cell that is introduced into the developing animal, and in the developmental stage of this introduction. In the second strategy, the cells giving rise to the cells, tissue(s), or organ(s) to be produced in the animal (*e.g.*, human cells) are introduced later in development, for example, at 45 to 60 days gestation in the cow, and the introduced cells are further differentiated than the embryonic stem cells used in the first strategy. For example, in the generation of a cow that produces human blood, human

hematopoietic stem cells (purified from individuals or derived from embryonic stem cells *in vitro*) are surgically introduced into a 45 to 60 day old fetus *in utero* that is in the process of developing from an unfertilized, enucleated egg that had undergone nuclear transfer with a cloning cell in which a gene or genes required for hematopoiesis had been knocked out. In the third strategy, both the donor cells, giving rise to the cells, tissue(s), or organ(s) to be produced in the animal (*e.g.*, human cells), and the cloning cells are introduced early in development, at the morula stage of embryo development (4 to 16 cell embryo). For all three strategies, the donor cells that give rise to the cells, tissue(s), or organ(s) to be produced in the animal (*e.g.*, human cells) can be produced by nuclear transfer into "reprogramming cells" of the same species (*e.g.*, unfertilized, enucleated human eggs). Alternatively, the reprogramming cells can be unfertilized, enucleated eggs of a different species, for example, bovine eggs (Lanza *et al.*, Nature Medicine 5:975-977, 1999). Each of these strategies, which include many common features, are described in further detail, as follows.

Animals in which cells, tissue(s), or an organ(s) of another organism (*e.g.*, a human) can be produced include, for example, mice, cows, sheep, pigs, and primates, such as chimpanzees, monkeys, and apes. Cloning cells, thus, are selected from any of these, or other, animals for use in the invention. Preferred cells from which cloning cells are derived are those that can be grown in tissue culture and that have stable chromosomes. For example, embryonic or fetal fibroblasts can be used to make the required genetic modifications (gene(s) knockout) for use as cloning cells. Cloning cells can also be derived from more specialized or differentiated cells, such as mammary gland cells in the ewe (Campbell *et al.*, *supra*). Other cells that can be used as cloning cells for genetic modification in the invention include, without limitation, adult fibroblast cells, cumulus cells, and muscle cells. It should be noted, however, that virtually any type of embryonic, fetal, or adult cell can be used as a cloning cell in the invention. The cell that is used for cloning by nuclear transfer can be quiescent, non-quiescent, or senescent (Campbell *et al.*, *supra;* Cibelli *et al.*, *supra*; Lanza *et al.*, Science 288:665-669, 2000).

Cells of an organism (*e.g.*, a human) that can be produced in an animal according to the invention include, *e.g.*, red blood cells, pancreatic islet cells, epithelial cells, neurons, and chondrocytes; the tissue(s) can be, *e.g.*, blood, the retina, or cartilage; and the organ(s) can be, *e.g.*, a pancreas, a heart, a liver, a kidney, intestine, a lung, or skin. Genes that can be knocked out to generate the animals of

the invention include, for example, GATA-2 (blood), LMO-2 (blood), globin genes (*e.g.*, α-globin and β-globin; blood), the erythropoietin receptor gene (erythroid cells; Wu *et al.*, Cell 83(1):59-67, 1995), PDX-1 (pancreas; Offield *et al.*, Development 122(3):983-985, 1996), and IPF-1 (Insulin promoter factor-1; pancreas; Jonsson *et al.*, Nature 371(6498):606-609, 1994). In essence, any gene required for the formation of a cell type, tissue, or organ can be knocked out according to the invention to produce a genetically modified cloning cell for production of the cell type, tissue, or organ of another organism (*e.g.*, a human) in an animal of the invention.

The genetically modified cloning cells used in the invention can be generated using standard methods for knocking out genes, such as homologous recombination (see, *e.g.*, Ausubel *et al.*, eds. Current Protocols in Molecular Biology, Wiley & Sons, New York, 1989) or mismatch repair using chimeric oligonucleotides (Cole-Strauss *et al.*, Science 273(5280):1386-1389, 1996).

In most embodiments of the invention, it is not necessary to replace a knocked out gene with another functional gene. This may be desired, however, under certain circumstances. For example, if a gene product encoded by a gene to be knocked out functions as a dimeric molecule (*e.g.*, a transcription factor), it may be desirable to knock out one allele of the gene and replace this allele with a gene encoding a dominant negative mutant of the gene product. In this case, it will not be necessary to knock out the other allele, as its function will be blocked by the dominant negative mutant produced from the introduced gene. If such dominant negative mutants are not used, it is necessary to knock out both alleles of a gene. This can be accomplished several ways. If the cells have a rather long replicative lifespan, both alleles can be sequentially knocked out to produce the cloning cell desired. If the cells have a relatively short replicative lifespan, one allele can be knocked out; a fetus is generated by nuclear transfer from these heterozygous knocked out cells; a new fibroblast cell line is generated lacking a single allele; and, finally, the second allele is knocked out, generating homozygous knock out cells that are then used as the cloning cells to make chimeric animals that produce the cells, tissue(s), or an organ(s) of another organism (*e.g.*, a human) as described above. Alternatively, the lifespan of a cloning cell can be increased by introduction of a constitutively expressed telomerase gene into the cell. Additionally, cloned fibroblasts that had been grown in culture until senescence have been shown to have an extended replicative lifespan after cloning (Lanza *et al.*, *supra*). Knocking out of several genes or alleles can be carried out in such cells, without the need for intermediate cloning steps. Also, it is possible, rather than to

knock out the coding sequences of a gene, to knock out only the control elements of the gene; of course, both the coding sequences and the control elements of a gene can be knocked out.

Introduction of the genetic material of a cloning cell, generated as described above, into a reprogramming cell is carried out using cloning or nuclear transfer methods, which have been described (see, *e.g.*, Campbell *et al.*, Nature 385:810-813, 1997; Wilmut, Scientific American, 58-63, December, 1998; Cibelli *et al.*, Science 280:1256-1258, 1998; Schnieke *et al.*, Science 278:2130-2133, 1997; Brown *et al.*, Science 277:831-834, 1997). Briefly, in these methods, a nucleated cell (referred to as the "cloning cell" in this application) is fused with a recipient unfertilized, enucleated, egg (referred to as a "reprogramming cell" in this application). Alternatively, only the nuclear genetic material of the donor cell is introduced into a recipient egg. The recipient egg can be taken from an animal soon after ovulation. Such eggs are poised to begin developing once they are appropriately stimulated. The egg can be held by suction on the end of a pipette under a high-power microscope, and an extremely fine micropipette can then be used to suck the chromosomes out from the egg creating an unfertilized, enucleated reprogramming cell.

Once the nuclear genetic material of the cloning cell has been introduced into the reprogramming cell, the egg is stimulated to develop by, for example, an electrical stimulus. The stimulated egg (1) is cultured into a morula for making chimeric morula using "donor" blastomeres, (2) is cultured to blastocysts for injection of "donor" embryonic stem cells (Thomson *et al.*, Science 282:1145-1147, 1998), or (3) is implanted into the uterus of a foster mother, for the surgical transplantation of "donor" stem cells at a later developmental stage (Kennedy *et al.*, Nature 386:488-493, 1998). In each of the above scenarios, the donor cells correspond to those cells, tissue(s), or organ(s) that are absent in the developing embryo/fetus because the gene (or genes) required for normal development and/or differentiation were knocked out in the cloning cells.

In the case of a blastocyst that is implanted directly into the uterus of a foster mother, the blastocyst is allowed to develop in the mother, and stem cells, such as human hematopoietic stem cells (Kennedy *et al.*, Nature 386:488-493, 1998), are surgically introduced into the developing fetus *in utero*, for example, at 45 to 60 days of gestation in the cow. It may be desirable to introduce different types of stem cells into the developing fetus in different anatomical places and/or different developmental stages, as will be readily understood by one of skill in this art. For

example, it may be desirable to inject hematopoietic stem cells into the fetus intravenously or to introduce these cells directly into the developing fetal liver.

Once a blastocyst or fetus has been obtained that contains the desired cells from another organism (*e.g.*, a human), pregnancy is allowed to proceed. The desired cells, tissue(s), and organ(s) can be harvested at any time during further embryonic or fetal development or after birth from juvenile or adult chimeric animals.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application. All references cited herein are incorporated by reference in their entirety. Other embodiments are within the following claims.

What is claimed is:

1. A non-human animal, or a precursor thereof, comprising cells, a tissue, or an organ, or a precursor thereof, of another organism, but not the corresponding cells, tissue, or organ, or precursor thereof, that would otherwise naturally occur in the animal.

2. The non-human animal of claim 1, wherein the other organism is a human.

3. The non-human animal of claim 1, wherein the animal is selected from the group consisting of a cow, a sheep, a pig, a mouse, or a primate, such as a chimpanzee, monkey, or ape.

4. The non-human animal of claim 1, wherein the cells are selected from the group consisting of red blood cells, pancreatic islet cells, epithelial cells, neurons, and chondrocytes; the tissue is selected from the group consisting of blood, the retina, and cartilage; or the organ is selected from the group consisting of a pancreas, a heart, a liver, a kidney, intestine, a lung, or skin.

5. The non-human animal of claim 1, wherein the animal is generated by knocking out the function of a gene encoding GATA-2, LMO-2, a globin, the erythropoietin receptor, PDX-1, or Insulin Promoter Factor-1.

6. A method of generating an animal comprising cells, a tissue, or an organ of another organism, but not the corresponding cells, tissue, or organ that would otherwise naturally occur in the animal, the method comprising:

a. Knocking out the function of a gene that is required for development of the cells, tissue, or organ in a cell of an animal to generate a genetically modified cloning cell;

b. Introducing nuclear genetic material of the cloning cell, or a derivative thereof, into a reprogramming cell from another organism, or fusing the cloning cell with the reprogramming cell;

c. Stimulating the resulting cell to develop into a blastocyst;

d. Introducing into the blastocyst donor embryonic stem cells of another organism; and

e. Implanting the resulting blastocyst into a pseudopregnant foster mother, where it develops into a chimeric animal that comprises cells, a tissue, or an organ that are derived from the donor embryonic stem cells, but not the corresponding cells, tissue, or organ that would otherwise naturally occur in the animal.

7. A method of generating an animal comprising cells, a tissue, or an organ of another organism, but not the corresponding cells, tissue, or organ that would otherwise naturally occur in the animal, the method comprising:

a. Knocking out the function of a gene that is required for development of the cells, tissue, or organ in a cell of an animal to generate a genetically modified cloning cell;

b. Introducing nuclear genetic material of the cloning cell, or a derivative thereof, into a reprogramming cell, or fusing the cloning cell with the reprogramming cell;

c. Stimulating the resulting cell to develop into an embryo;

d. Implanting the embryo into a pseudopregnant foster mother; and

e. Introducing donor stem cells of another organism into the developing embryo or fetus *in utero*, so that the resulting embryo or fetus develops into a chimeric animal that comprises cells, a tissue, or an organ derived from the donor stem cells, but not the corresponding cells, tissue, or organ that would otherwise naturally occur in the animal.

8. A method of generating an animal comprising cells, a tissue, or an organ of another organism, but not the corresponding cells, tissue, or organ that would otherwise naturally occur in the animal, the method comprising:

a. Knocking out the function of a gene that is required for development of the cells, tissue, or organ in a cell of an animal to generate a genetically modified cloning cell;

b. Introducing nuclear genetic material of the cloning cell, or a derivative thereof, into a reprogramming cell, or fusing the cloning cell with the reprogramming cell;

c. Stimulating the resulting cell to develop into a morula;

d. Disaggregating individual blastomeres of the morula;

e. Constructing a chimeric morula by injecting the disaggregated blastomeres and disaggregated donor blastomeres of another organism into a zona pellucida or by aggregating the blastomeres of the two organisms; and

f. Implanting the reconstructed morula into a pseudopregnant foster mother, where it develops into a chimeric animal that produce cells, a tissue, or an organ derived from the donor blastomeres, but not the corresponding cells, tissue, or organ that would otherwise naturally develop in the animal.

9. The method of claim 6, 7, or 8, wherein the cloning cell is an embryonic, fetal, or adult fibroblast.

10. The method of claim 6, 7, or 8, wherein the reprogramming cell is an unfertilized enucleated egg from another organism of the same, or a different, species from which the cloning cell was obtained.

11. The method of claim 6, 7, or 8, wherein the function of the gene is knocked out by homologous recombination.

12. The method of claim 6, 7, or 8, wherein the nuclear genetic material of the cloning cell is introduced into the reprogramming cell by nuclear transfer.

13. The method of claim 6, wherein the donor cells are human embryonic stem cells.

14. The method of claim 7, wherein the donor cells are human stem cells.

15. The method of claim 8, wherein the donor blastomeres are human blastomeres.

16. A method of treating a patient in need of a transplant of a cell, tissue, or organ, comprising transplanting into the patient cells, a tissue, or an organ produced in the animal of claim 1 or by the method of claim 6, 7, or 8.

17. A cell, tissue, or organ produced by the method of claim 6, 7, or 8.

18. Use of a cell, tissue, or organ produced by the method of claim 6, 7, or 8 in the treatment of a patient in need of a transplant of a cell, tissue, or organ, by transplanting the cell, tissue, or organ into the patient.

19. Use of a cell, tissue, or organ produced by the method of claim 6, 7, or 8 in the preparation of a medicament for treatment of a patient in need of a transplant of the cell, tissue, or organ.

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

(X) Original ( ) Supplemental ( ) Substitute ( ) PCT

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **"PRODUCTION OF HUMAN CELLS, TISSUES, AND ORGANS IN ANIMALS"**, which is described and claimed in the specification

(check one) [ ] which is attached hereto, or
[X] which was filed on November 13, 2001, as United States Application No. 10/009,359 and with amendments (if applicable), or
[ ] in International Application No. PCT/, filed , and as amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information known by me to be material to the patentability of the claims of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code §119 (a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) or §365(b) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATIONS: (ENTER BELOW IF APPLICABLE)			PRIORITY CLAIMED (MARK APPROPRIATE BOX BELOW)	
APP. NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	YES	NO
PCT/US00/12953	PCT	12 May 2000	X	

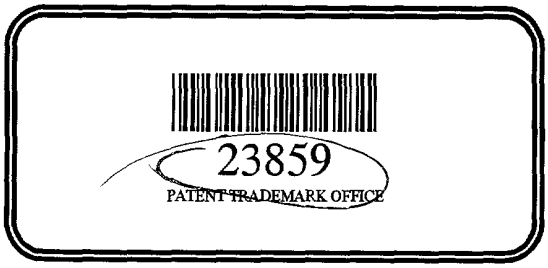
I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

APPLICATION NUMBER	FILING DATE
60/133,935	13 May 1999

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information known by me to be material to the patentability of the claims of this application as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS (MARK APPROPRIATE COLUMN BELOW)		
		PATENTED	PENDING	ABANDONED

I hereby appoint the following attorneys and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:



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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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